

purified with Sep-pak Vac(C18) in accordance with a similar manner to that of Example 6, to obtain 12.4 mg of Leu-Tyr-Gln-Ala-Val-Ala-Thr-Ile (SEQ ID NO: 5).

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The peptide obtained, Leu-Tyr-Gln-Ala-Val-Ala-Thr-Ile (SEQ ID NO: 5), had a retention time of 19.2 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6 ϕ x 250 mm) eluted with a linear gradient of acetonitrile concentration from 0 to 60% containing 0.1% TFA, and the results of amino acid analysis and mass spectrometry of the product were consistent with the theoretical values.

Please replace the Sequence Listing of record with the Substitute Sequence Listing enclosed herewith.

REMARKS

Enclosed herewith is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification. Also submitted herewith is a computer readable form of the Substitute Sequence Listing. The computer readable form of the Substitute Sequence Listing, file "0020-4872P.ST25.txt", is identical to the paper copy, except that it lacks formatting.

The paragraph changes made by this amendment are intended to reference each amino acid sequence by a SEQ ID NO. and do not add new matter to the specification. Attached hereto is a

Application No. 09/857,308

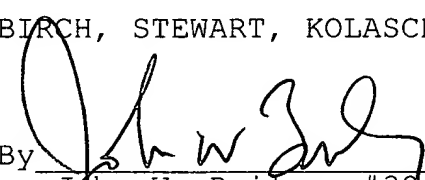
marked-up version of the changes made to the specification by this amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. \$1.16 or under 37 C.F.R. \$1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By



John W. Bailey, #32,881
P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

JWB/BCF

Enclosures: Paper and Disk Copy of the Substitute Sequence
Listing, Copy of the Notice to Comply, Version with
Markings

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The paragraph beginning on page 50 line 10 and ending on page 51 line 1:

To this peptide resin, 1 ml of Reagent K (the solution of 5% phenol, 5% thioanisole, 5% H₂O, and 2.5% ethanedithiol in TFA) was added and the mixture was allowed to react for 2.5 hours at room temperature. While cooling with ice, 10 ml of diethyl ether was added to the reaction, the mixture was stirred for 10 minutes, filtered, and washed with 10 ml of diethyl ether. To the filter cake, 10 ml of aqueous acetic acid was added, and the mixture was stirred for 30 minutes. The resin was then filtered, and washed with 4 ml of aqueous acetic acid. After lyophilizing the filtrate and the wash, the crude peptide obtained was dissolved in aqueous acetic acid, and injected into a reverse phase packing material, YMC-PACK ODS-A SH-363-5 column (30 ϕ x 250 mm) that had been pre-equilibrated with 0.1% aqueous TFA. The column was washed with 0.1% aqueous TFA, and elution at a flow rate of 7 ml/min was then conducted, while increasing the concentration of acetonitrile up to 40% over 240 minutes. The eluate was monitored by A 220 nm. The fractions containing the desired product were combined together and lyophilized to obtain 15.4 mg of Gly-Phe-Asp-Cys-Ala-Asn-Glu-Ser-Val-Leu (SEQ ID NO: 3).

The paragraph beginning on page 51 line 2:

The peptide obtained, Gly-Phe-Asp-Cys-Ala-Asn-Glu-Ser-Val-Leu (SEQ ID NO: 3), had a retention time of 19.9 minutes in an analysis using a reverse phase packing material, YMC-PACK ODS-AM AM-303 column (4.6 ϕ x 250 mm)

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eluted with a linear gradient of acetonitrile concentration from 0 to 60% containing 0.1% TFA, and the results of amino acid analysis (Cys being not detected) and mass spectrometry of the product were consistent with the theoretical values.

The paragraph beginning on page 52 line 18 and ending on page 53 line 5:

According to a similar manner to that described in Example 5, using 50 mg of Fmoc-Leu-Alko Resin (0.57mmol/g, 100-200mesh), Fmoc-Lys(Boc)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Cys(Trt)-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Glu(OtBu)-OH were coupled in order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid. The solution was separated into two portions, and each was purified with Sep-pak Vac (C18). Specifically, each portion was injected into the cartridge that had been pre-equilibrated with 0.1% aqueous TFA. The cartridge was washed three times with 10ml of 0.1% aqueous TFA, and was eluted three times with 10 ml of 0.1% aqueous TFA-acetonitrile (1:1). The eluate was collected and lyophilized to obtain 37.5 mg of Glu-Tyr-Cys-Leu-Lys-Phe-Thr-Lys-Leu (SEQ ID NO: 4).

The paragraph beginning on page 53 line 6:

The peptide obtained, Glu-Tyr-Cys-Leu-Lys-Phe-Thr-Lys-Leu (SEQ ID NO: 4), had a retention time of 20.8 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM-303 column (4.6 ϕ x 250 mm) eluted with a linear gradient of acetonitrile concentration from 0 to 60%

containing 0.1% TFA, and the results of amino acid analysis (Cys being not detected) and mass spectrometry of the product were consistent with the theoretical values.

The first and second full paragraphs on page 54 beginning with line 3 and ending with line 17:

According to a similar manner to that described in Example 5, using 50 mg of Fmoc-Ile-Alko Resin (0.62mmol/g, 100-200mesh), Fmoc-Thr(tBu)-OH, Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Ala-OH, Fmoc-Gln-OH, Fmoc-Tyr(tBu)-OH, and Fmoc-Leu-OH were coupled in order, and the product was then deprotected. The crude peptide obtained was dissolved in aqueous acetic acid and purified with Sep-pak Vac(C18) in accordance with a similar manner to that of Example 6, to obtain 12.4 mg of Leu-Tyr-Gln-Ala-Val-Ala-Thr-Ile (SEQ ID NO: 5).

The peptide obtained, Leu-Tyr-Gln-Ala-Val-Ala-Thr-Ile (SEQ ID NO: 5), had a retention time of 19.2 minutes in an analysis using a reverse phase packing material YMC-PACK ODS-AM column (4.6 ϕ x 250 mm) eluted with a linear gradient of acetonitrile concentration from 0 to 60% containing 0.1% TFA, and the results of amino acid analysis and mass spectrometry of the product were consistent with the theoretical values.

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